

## In Vitro Studies of Netilmicin, a New Aminoglycoside Antibiotic

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Netilmicin, a semisynthetic derivative of sisomicin, was tested in vitro against 600 clinical bacterial isolates. At a concentration of 1.56  $\mu\text{g/ml}$ , over 90% of gram-negative bacilli were inhibited. Netilmicin was substantially more active against isolates of *Serratia marcescens* and *Enterobacter* spp. than gentamicin, sisomicin, tobramycin, or amikacin. Isolates of *Staphylococcus aureus* (both penicillin G susceptible and resistant) were quite susceptible to netilmicin. Most isolates of *Klebsiella* spp. and *Serratia* spp. and some of the isolates of *Pseudomonas aeruginosa* that were resistant to gentamicin proved to be susceptible to netilmicin.

Gram-negative bacilli are responsible for an increasing number of infections in hospitalized patients (2). Aminoglycoside antibiotics, such as gentamicin, amikacin, and tobramycin, have been quite successful in the treatment of gram-negative infections. However, increased resistance to these antibiotics has developed in recent years (6, 7). Recently, a new aminoglycoside, netilmicin, has been synthesized from sisomicin, which is produced by *Micromonospora inyoensis*. It has a spectrum of in vitro activity that is similar to gentamicin sulfate. We determined the activity of netilmicin against clinical bacterial isolates and compared its activity with that of gentamicin, sisomicin, tobramycin, and amikacin. The activity of netilmicin was also determined against gentamicin-resistant isolates of gram-negative bacilli.

### MATERIALS AND METHODS

Susceptibility tests were conducted on 497 gram-negative bacilli and 103 gram-positive cocci, using the dilution technique with an automatic microtiter system (*Autotiter Instruction Manual*, Canalco). All isolates of gram-negative bacilli and *Staphylococcus aureus* were inoculated into Mueller-Hinton broth (Difco) and incubated at 37°C for 18 h. Other gram-positive cocci were inoculated into tryptose phosphate broth. An inoculum of approximately  $10^8$  colony-forming units/ml was used to test isolates of gram-negative bacilli and *S. aureus*. For the other gram-positive cocci, an inoculum of  $10^8$  colony-forming units/ml was used for susceptibility testing.

All gram-negative bacilli used in this study were cultured from blood specimens obtained from patients hospitalized at The University of Texas M. D. Anderson Hospital between the years 1967 and 1976. The majority of these patients had underlying ma-

lignant diseases. A total of 100 isolates each of *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella* spp., and *Enterobacter* spp., 64 isolates of *Proteus* spp., and 50 isolates of *Serratia marcescens* were utilized. All gram-positive cocci used in this study were cultured from hospitalized patients, most of whom did not have underlying malignant diseases. A total of 41 isolates of *Streptococcus pyogenes*, 12 isolates of *S. pneumoniae*, and 50 isolates of *S. aureus* were studied. The susceptibility of isolates of *S. aureus* to penicillin G was determined by means of the broth dilution technique. Those isolates inhibited by less than 0.10  $\mu\text{g/ml}$  were considered to be penicillin G susceptible, and those isolates resistant to more than 25  $\mu\text{g/ml}$  were considered to be resistant to penicillin G. Isolates of gram-negative bacilli that had minimum inhibitory concentrations (MIC) of 12.5  $\mu\text{g}$  or greater per ml were considered to be resistant to gentamicin.

Netilmicin, sisomicin, and gentamicin were supplied by Schering Corp., Bloomfield, N.J. Tobramycin and amikacin were supplied by Eli Lilly & Co., Indianapolis, Ind., and Bristol Laboratories, Division of Bristol-Myers Co., Syracuse, N.Y., respectively. Twofold serial dilutions of the antibiotics were made with appropriate broth, and the MIC was determined after incubation at 37°C for 18 h. The minimum bactericidal concentration (MBC) was defined as the lowest concentration of drug that yielded less than 25 colonies when 0.01 ml was subcultured onto sheep blood agar (less than five colonies per 0.001 ml of inoculum). A calibrated pipette was used to transfer the inoculum. All studies were performed in triplicate, and comparative studies were done simultaneously.

### RESULTS

The in vitro activity of netilmicin against gram-negative bacilli and gram-positive cocci is

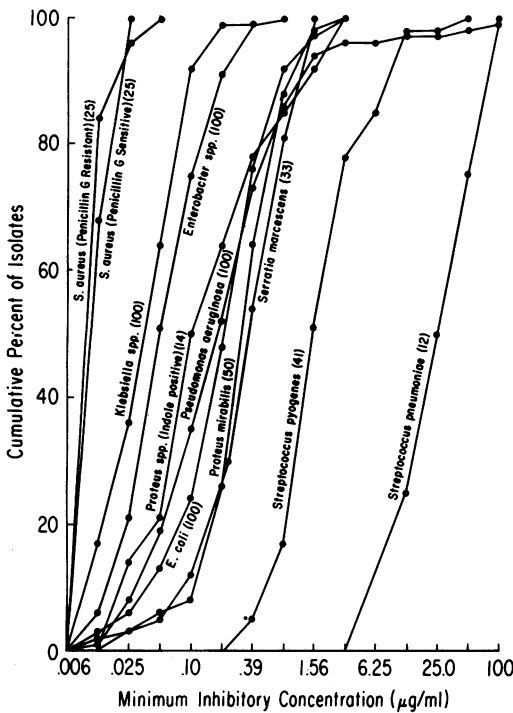


FIG. 1. *In vitro* activity of netilmicin.

shown in Fig. 1. The MIC of netilmicin was 1.56 µg or less per ml against all isolates of *Klebsiella* spp., *S. marcescens*, and *Enterobacter* spp., 97% of *E. coli*, 97% of *Proteus mirabilis*, 92% of indole-positive *Proteus* spp., and 94% of *P. aeruginosa*. All penicillin G-susceptible and penicillin G-resistant isolates of *S. aureus* strains were susceptible to netilmicin at a concentration of 0.05 µg or less per ml. Netilmicin inhibited 78% of the isolates of *S. pyogenes* at a concentration of 3.12 µg/ml, whereas against *S. pneumoniae* it had no activity at this concentration.

In general, the MBC of most of the clinical isolates, with the exception of the *S. marcescens* isolates, equaled the MIC or was a twofold dilution greater. For example, the MBC was 1.56 µg/ml against 94% of isolates of *E. coli*, whereas the MIC was 0.78 µg/ml against 92%. For isolates of *S. marcescens*, the MBC was six times greater than the MIC.

Figure 2 shows a comparison of the *in vitro* activity of netilmicin with that of four other aminoglycosides: gentamicin sulfate, tobramycin, sisomicin, and amikacin against *Enterobacteriaceae*. The activity of netilmicin was similar to that of gentamicin sulfate. Against isolates of *S. marcescens* and *Enterobacter*

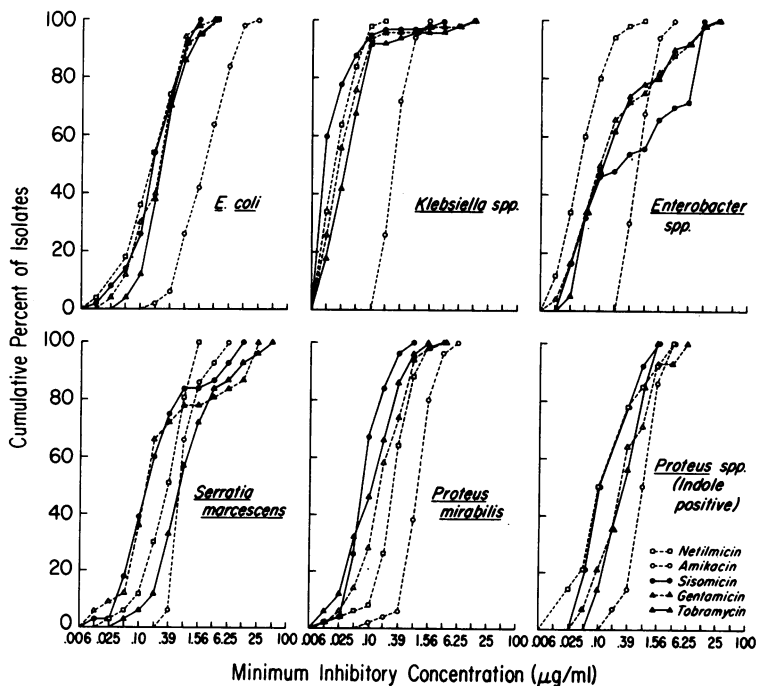


FIG. 2. Comparative activity of aminoglycosides against gram-negative bacilli.

spp., it was substantially more active than the other aminoglycosides. At a concentration of 1.56  $\mu\text{g/ml}$ , netilmicin inhibited all isolates of *S. marcescens*, whereas amikacin, sisomicin, gentamicin, and tobramycin inhibited 87, 84, 78, and 72%, respectively. All isolates of *Enterobacter* spp. were susceptible to netilmicin at a concentration of 0.78  $\mu\text{g/ml}$ . Amikacin and sisomicin showed the least activity against *Enterobacter* spp. Sisomicin, gentamicin, tobramycin, and netilmicin had similar activity against *Klebsiella* spp. All isolates were inhibited by netilmicin at a concentration of 0.20  $\mu\text{g/ml}$ . Netilmicin inhibited 92% of the isolates of indole-positive *Proteus* spp. at a concentration of 1.56  $\mu\text{g/ml}$ , whereas sisomicin and tobramycin inhibited 100% of these isolates at the same concentration. Netilmicin was slightly more active against isolates of *P. mirabilis*, inhibiting 98% at this concentration.

The in vitro susceptibility of 50 isolates of *P. aeruginosa* to the five aminoglycosides is shown in Fig. 3. At a concentration of 0.20  $\mu\text{g/ml}$ , sisomicin and tobramycin were the most active, both inhibiting 88% of the isolates. At the same concentration, netilmicin, gentamicin, and amikacin inhibited 70, 78, and 12%, respectively. All of the aminoglycosides inhibited over 90% of the isolates at a concentration of 3.12  $\mu\text{g/ml}$ .

Twenty-three isolates of gram-negative bacilli known to be resistant to gentamicin, tobramycin, or sisomicin were tested for their susceptibility to netilmicin (Table 1). All six isolates of *Klebsiella* spp. were susceptible to netilmicin and amikacin. Some of these isolates were considerably more susceptible to netilmicin than to amikacin. Netilmicin was active against 80% of the isolates of *Serratia* spp. Two isolates of *Serratia* spp. were susceptible only to amikacin. Of the seven *Pseudomonas* isolates tested, four were equally susceptible to netilmicin and amikacin, two were resistant to

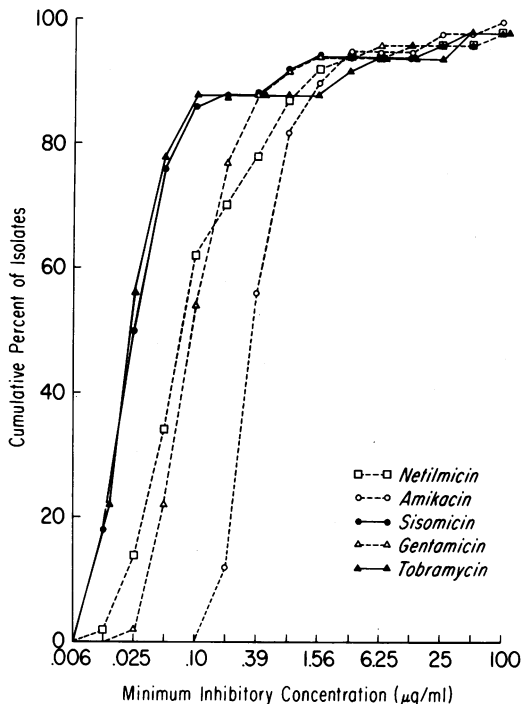


FIG. 3. Comparative activity of antibiotics against *P. aeruginosa*.

netilmicin but susceptible to amikacin, and one was resistant to all five aminoglycosides.

### DISCUSSION

Netilmicin is an aminoglycoside antibiotic with a broad-spectrum of activity against gram-negative bacilli and some gram-positive cocci. The in vitro activity of this antibiotic against gram-negative bacilli is similar to that of gentamicin sulfate. However, netilmicin was more active than other aminoglycosides against isolates of *Klebsiella* spp., *Enterobacter* spp., and

TABLE 1. Activity of aminoglycosides against gram-negative bacilli resistant to gentamicin sulfate

Organism	No. of isolates	MIC ( $\mu\text{g/ml}$ ) for:									
		Amikacin		Sisomicin		Gentamicin		Tobramycin		Netilmicin	
		Median	Range	Median	Range	Median	Range	Median	Range	Median	Range
<i>Klebsiella</i> spp.	6	0.20	0.10-0.39	3.12	3.12-6.25	12.5	12.5-25	12.5	12.5-50	0.025	0.012-0.39
<i>P. aeruginosa</i>	7	0.39	0.10-100	>100	50->100	>100	100->100	50	50->100	0.39	0.20->100
<i>S. marcescens</i>	10	1.56	0.78-12.5	25	6.25-50	50	25-100	50	25-100	1.56	1.56-100

*S. marcescens*. Also, it was as active as gentamicin and tobramycin against *E. coli* isolates. It was not as active as sisomicin or tobramycin against isolates of *P. aeruginosa*.

Our results are in general agreement with other investigations (3, 5). However, most of our isolates of *Pseudomonas* spp. were equally susceptible to netilmicin and gentamicin. Miller et al. found netilmicin to be twofold less active than gentamicin. This difference could be explained by the different methods used in determination of the MIC (5). We found most isolates of gentamicin-resistant strains of *Pseudomonas* spp. to be susceptible to netilmicin. Some isolates of *Klebsiella* spp. resistant to gentamicin were more susceptible to netilmicin than to amikacin. Most isolates of *S. marcescens* were equally susceptible to both antibiotics. Miller et al. also found that some of their strains of *Enterobacteriaceae* were considerably more susceptible to netilmicin.

Netilmicin appears to have some advantages over other aminoglycoside antibiotics, such as gentamicin, tobramycin, amikacin, and sisomicin. Because of its greater in vitro activity against some clinical isolates of gram-negative bacilli, it deserves further investigation.

#### ACKNOWLEDGMENTS

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